



Caspase-3 activity is present in cerebrospinal fluid from patients with traumatic brain injury

Luc Härter ^{a,*}, Marius Keel ^a, Hannes Hentze ^b, Marcel Leist ^c, Wolfgang Ertel ^a

^a Department of Trauma Surgery, University Hospital Zürich, Zürich, Switzerland

^b Institute of Molecular and Cell Biology, 117609 Singapore, Singapore

^c H. Lundbeck A/S, 2500 Valby, Denmark

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Abstract

Loss of neurons after traumatic brain injury (TBI) might involve dysregulated apoptosis. Activation of caspase-3 is one hallmark of apoptosis. Therefore, caspase-3 activity (cleavage of DEVD-afc) was measured in cerebrospinal fluid (CSF) samples ($n = 113$) from 27 patients with TBI at day 1 to 14 after trauma. Caspase-3 activity was detected in 31 (27.4%) CSF samples with highest values ($> 5.5 \mu\text{M}/\text{min}$) seen at day 2–5 after trauma. No caspase-3 activity was found in serum from patients or CSF from controls. The presence of activated caspase-3 in CSF suggests ongoing apoptotic processes during traumatic brain injury. © 2001 Elsevier Science B.V. All rights reserved.

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Neuronal cell loss after traumatic brain injury (TBI) has been formerly attributed to necrosis of neurons as a result of secondary insults. More recent evidence from animal models (Rink et al., 1995) and findings in human patient tissues (Ng et al., 2000; Clark et al., 1999) point towards an involvement of apoptosis in the mechanism of neuronal destruction following TBI. Apoptosis in neurons, endothelial cells, and astrocytes is triggered through the CD95 system (Leist and Nicotera, 1998). Because high concentrations of CD95-ligand were found in cerebrospinal fluid (CSF) from patients with severe TBI (Ertel et al., 1997), this points to the involvement of apoptotic processes responsible for brain damage following TBI.

A group of intracellular cysteine proteases, called caspases has been shown to play a pivotal role in the regulation and execution of apoptosis. Within this group, caspase-3 is the main executioner protease and its activation marks a point-of-no-return in the complicated cascade of apoptosis induction. Thus, the presence of active caspase-3 is a good indicator of apoptosis. To date, it is unknown whether active caspase-3 can be released from cells dying by apoptosis into CSF following TBI.

Therefore, caspase-3 activity was examined in the CSF of 27 patients with severe TBI. All patients were treated

according to our standard protocol for TBI (Stocker et al., 1995), which was approved by the University Hospital Medical Ethics Board. Three patients (11.1%) succumbed to death due to TBI. As a control, the CSF from seven patients requiring spinal anesthesia for elective orthopedic surgery (with normal CSF protein content and cell count) were included in this study after written informed consent. The control group was comparable to the trauma patients with regard to age and gender (Table 1).

CSF drained from an indwelling ventricular catheter was collected from all patients each day at 8 am between days 1 and 14 after trauma. However, in some patients, it was not possible to obtain CSF on consecutive days due to failure of the intraventricular catheter, collapsed ventricles due to extensive brain edema or death of the patient. The preflow (0.5 ml) was discarded and 1–2 ml of the sterile CSF centrifuged ($300 \times g$, 10 min, 4 °C) and then frozen immediately at -80 °C until further processing.

Caspase-3 activity in the CSF was measured by a fluorimetric assay based on the specific hydrolysis of DEVD-7-amino-4-trifluoromethylcoumarin (DEVD-afc, 60 μM) in substrate buffer (50 mM HEPES, pH 7.4, 1% sucrose, 0.1% Chaps, 10 mM DDT, Thornberry, 1994). Caspase-3 activity (μM afc/min) was determined by measuring the increase of afc-fluorescence (excitation at 385 nm; fluorescence emission at 505 nm) over 30 min at 37 °C, and calculation of the afc formation/time based on an

* Corresponding author.